Indole-2-carboxamide Inhibitors of Human Liver Glycogen Phosphorylase†

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Type 2 diabetes (formerly non-insulin-dependent diabetes mellitus) is a severe and prevalent disease. Diabetics may suffer debilitating eye, kidney, and nerve damage and are at risk of premature death due to these and other diabetic complications.[1] Tight control of plasma glucose, achieved by intensive insulin therapy, reduces the incidence and progression of diabetic complications in type 1 and type 2 diabetics.[2,3] Unfortunately, achieving control is often impossible with current oral hypoglycemic agents. Moreover, their chronic use often brings, in addition to toleration, fear of dangerous hypoglycemic episodes. Safer and more effective therapy is urgently needed.

We targeted liver glycogen phosphorylase (EC 2.4.1.1) as an approach to controlling elevated glucose levels. The liver is the predominant source of plasma glucose in fasting type 2 diabetics.[4−7] Figure 1 shows, schematically, selected details of hepatic glucose synthesis, storage, and release.[8] Glycogen phosphorylase[9,10] releases glucose-1-phosphate from glycogen. Although significant evidence exists of an important role for glycogenolysis in hepatic glucose production,[11−14] glycogen phosphorylase is not a widely pursued antidiabetic target, partly because of conclusions that the high glucose levels in type 2 diabetics arise independently from elevated gluconeogenesis.[7,15,16] Recent data, however, showing that much of glucose arising from gluconeogenesis has cycled through glycogen,[17] exposes a possibility that inhibition of glycogen phosphorylase could suppress hepatic glucose arising from both pathways.[18] Orally active glycogen phosphorylase inhibitors[19] would clearly be valuable additions to this research area.

Activity of liver glycogen phosphorylase is regulated physiologically by hormonal and neuronal signals which alter phosphorylation at serine 14. Only the phosphorylated enzyme has significant activity. The liver isozyme is also regulated by ligands which bind to a distant allosteric site, termed the nucleotide activation site. AMP binds here as an activator,[20] while ATP and

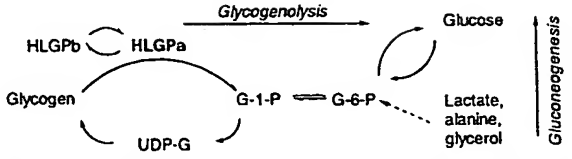

Figure 1. Selected details of hepatic glycogenolysis and gluconeogenesis. Abbreviations: HLGP*a*, human liver glycogen phosphorylase *a* (phosphorylated, active form); HLGP*b*, unphosphorylated form; UDP-G, uridine diphosphoglucose; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate.

glucose-6-phosphate (G-6-P) bind here as inhibitors.[21] Synthetic G-6-P analogues[22] and a dihydropyridinedicarboxylic acid[23] also inhibit at the nucleotide activation site of rabbit muscle glycogen phosphorylase *b* (RMGP*b*). Most reported phosphorylase inhibitors[10] are glucose or other sugar-based analogues,[24] which have in numerous instances been shown to bind at the catalytic site of RMGP*b*.[25−29] Caffeine and other nucleosides inhibit at another allosteric site, termed the purine inhibitor site.[30] No physiologic role or ligand for the purine inhibitor site is known, although xanthines may regulate phosphorylase activity here in certain physiologic states.[31,32] Inhibition by caffeine and glucose is synergistic, because of cooperative ordering of both binding sites.[25] This is an attractive property, for a therapeutic inhibitor which would lose potency as glucose levels fall should offer reduced risk of severe hypoglycemia.

Recently, we reported finding the orally active glycogen phosphorylase inhibitor **1** (Table 1) by high-throughput screening against recombinant human liver glycogen phosphorylase *a* (rHLGP*a*).[18] Other inhibitors of the human liver isoform[33] of glycogen phosphorylase have not been described. Here, we demonstrate that compounds in two series derived from **1** (Tables 1 and 2) are also potent inhibitors of rHLGPa and have cellular and oral activity. Their synthesis and characterization are also described.

The simple, heterocyclic, and asymmetric structure of **1** was exciting, as was the opportunity to explore the importance of these features to its high inhibitory activity. Inhibitor **1** and other (3*S*,2*R*)-2-amino-3-hydroxy-4-phenylbutyric acid derivatives were prepared as shown in Scheme 1. Methyl ester **28** was formed on treatment of cyanohydrin **27**[34] with anhydrous HCl in methanol and purified by extraction and recrystallization. Carbodiimide-mediated coupling with commercially available indole-2-carboxylic acids gave methyl ester **10** and the fluoro, bromo, and deschloro analogues **29**−**31** in high yield. Some of these products contained up to 10% of the diester resulting from hydroxyl acylation, and were hydrolyzed with aqueous sodium hydroxide in methanol, giving acids **11** and **32**−**34** in pure form after extraction and trituration. Coupling with dimethylamine and methylamine hydrochlorides gave target amides **1** and **5**−**8** in good yields. The stereoisomers of **1** (**2**−**4**) were also synthesized this way, from the stereoisomers of methyl ester **28**, which were obtained as their hydrochlorides in quantitative yield by refluxing the commercial (2*S*,3*R*)-, (2*R*,3*R*)-, and

† This manuscript is dedicated to the occasion of Professor E. J. Corey's 70th birthday.

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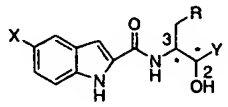
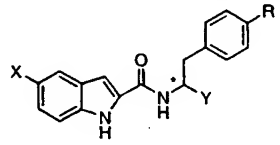
Table 1. Structures and in Vitro Phosphorylase Inhibitory Activity of 3-[(Indole-2-carbonyl)amino]-2-hydroxy-4-phenylbutyric Acid Analogues



compd	X	R	Y	*	rHLGP*a* IC_{50}, nM[a]	cell IC_{50}, μM[b]
1	Cl	Ph	$CONMe_2$	3*S*,2*R*	110 ± 12	1.5
2	Cl	Ph	$CONMe_2$	3*R*,2*S*	1800 ± 320	
3	Cl	Ph	$CONMe_2$	3*R*,2*R*	1700 ± 190	
4	Cl	Ph	$CONMe_2$	3*S*,2*S*	8700 ± 1700	
5	F	Ph	$CONMe_2$	3*S*,2*R*	170 ± 16	6.2
6	Br	Ph	$CONMe_2$	3*S*,2*R*	97 ± 11	3.3
7	H	Ph	$CONMe_2$	3*S*,2*R*	440 ± 48	>30
8	Cl	Ph	CONHMe	3*S*,2*R*	680 ± 37	>30
9	Cl	Ph	$CONH_2$	3*S*,2*R*	1700 ± 140	
10	Cl	Ph	COOMe	3*S*,2*R*	210 ± 40	13
11	Cl	Ph	COOH	3*S*,2*R*	330 ± 31	>30
12	Cl	Ph	CH_2OH	3*S*,2*R*	6800 ± 1700	
13	Cl	Cy[c]	$CONMe_2$	3*S*,2*R*	6500 ± 610	

[a] Arithmetic mean ± SEM ($n \geq 3$, each in triplicate). [b] Inhibition of forskolin-stimulated glycogenolysis in SK-HEP-1 cells (pooled data from $n \geq 2$, each in triplicate). [c] Cyclohexyl.

Table 2. Structures and in Vitro Phosphorylase Inhibitory Activity of *N*-(Indole-2-carbonyl) Phenylalanine Analogues



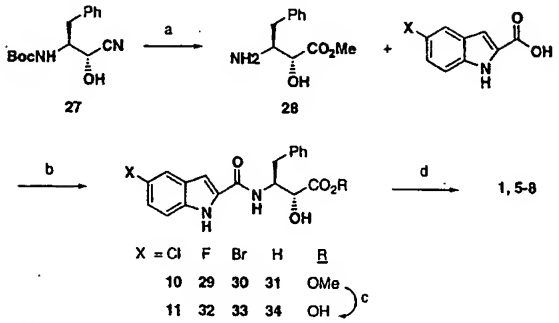
compd	X	R	Y	*	rHLGP*a* IC_{50}, nM[a]	cell IC_{50}, μM[b]
14	Cl	H	$CONMe_2$	*S*	82 ± 10	1.9
15	Cl	H	$CONMe_2$	*R*	260 ± 33	12
16	Cl	H	CONHMe	*S*	110 ± 10	5.2
17	Cl	H	CONHMe	*R*	220 ± 32	12
18	Br	H	$CONMe_2$	*S*	110 ± 8	1.4
19	F	H	$CONMe_2$	*S*	430 ± 44	2.3
20	H	H	$CONMe_2$	*S*	400 ± 45	7.8
21	OMe	H	$CONMe_2$	*S*	4700 ± 300	
22	Cl	H	CO(1-piperidin-4-ol)	*S*	260 ± 17	5.1
23	Cl	F	CO(1-piperidin-4-ol)	*S*	205 ± 16	1.9
24	Cl	H	COOMe	*S*	140 ± 18	>30
25	Cl	H	COOH	*S*	1700 ± 490	>30
26	Cl	H	CH_2OH	*S*	>10000[c]	

[a] Arithmetic mean ± SEM ($n \geq 3$, each in triplicate). [b] Inhibition of forskolin-stimulated glycogenolysis in SK-HEP-1 cells (pooled data from $n \geq 2$, each in triplicate). [c] 23% inhibition at 10 μM.

(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acids with chlorotrimethylsilane in methanol.[35] Less than 1% of **1** was present in isomers **2**–**4** by chiral stationary phase HPLC analysis. Primary amide **9** was obtained by heating methyl ester **10** with ammonia in methanol. Diborane reduction of acid **11** gave diol **12**. Cyclohexane **13** was prepared by steps b–d of Scheme 1, starting from (2*R*,3*S*)-3-amino-4-cyclohexylmethyl-2-hydroxybutyric acid isopropyl ester.[36]

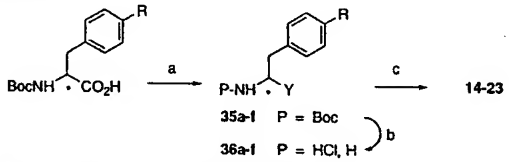
Phenylalanine-containing analogues of **1** were also synthesized (Scheme 2), by stepwise coupling from the C-terminus using *t*-Boc-protected phenylalanines. Methyl ester **24** and alcohol **26** were obtained from L-phenylalanine methyl ester hydrochloride and L-phenylalaninol, respectively. Hydrolysis of **24** with aqueous lithium hydroxide in THF gave acid **25**. *N*-Methylindole **37** and benzofuran **38** were obtained from (*S*)-phenylalanine dimethylamide hydrochloride (**35a**) and 1-methylindole-2-carboxylic acid or 5-chlorobenzofuran-2-carboxylic acid.

Scheme 1[a]

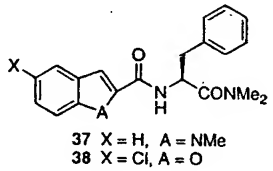


[a] Key: (a) HCl–MeOH, 60 h, 23 °C, then $NaHCO_3/H_2O$, 77%; (b) 1.0 equiv of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, 1.5 equiv of 1-hydroxybenzotriazole, dichloromethane, 23 °C, 18 h; (c) 2.0 equiv of NaOH, MeOH, H_2O, 25 °C, 2 h; (d), as for (b), but using also 1.1 equiv of either $Me_2NH{\cdot}HCl$ or $MeNH_2{\cdot}HCl$ and 1.1 equiv of triethylamine in DMF, 18 h, 23 °C.

Scheme 2[a]



[a] R, Y, and X are defined in Table 2. Key: (a) 1.0–1.1 equiv of dimethylamine, methylamine, or 4-hydroxy-1-piperidine hydrochloride, 1.1 equiv of Et_3N, 1.0 equiv of DEC, 1.5 equiv of HOBT, CH_2Cl_2, 0–20 °C, 18 h; (b) HCl–dioxane, 23 °C, 1 h; (c) 1.0 *X*-indole-2-COOH, 1.0 DEC, 1.5 HOBT, 1.0 Et_3N, CH_2Cl_2, 23 °C, 18 h.



Compounds were assessed for ability to inhibit rHLGP*a*-catalyzed release of phosphate from glucose-1-phosphate (reverse direction of the physiological reaction) in the presence of glycogen and a high physiological concentration of glucose (7.5 mM).[18] The (3*S*,2*R*) stereochemistry of **1** is optimal as seen in the reduced inhibitory activity of stereoisomers **2**-**4** (Table 1). Inhibitory activity was also sensitive to changes in the indole 5-substituent. Activity was completely retained in bromo analogue **6**, but diminished 1.5- and 4-fold in the 5-fluoro and deschloro analogues (**5** and **7**, respectively). C-Terminally modified analogues showed considerable variation in their inhibitory activity. Monomethylamide **8** and primary amide **9** showed 6- and 15-fold lower activity than **1**. The synthetic intermediates **10** (methyl ester) and **11** (carboxylic acid) were closer in activity to **1**. Diol **12** showed about a 60-fold reduction of inhibitory activity relative to **1**, as did the cyclohexylmethyl side chain analogue **13**.

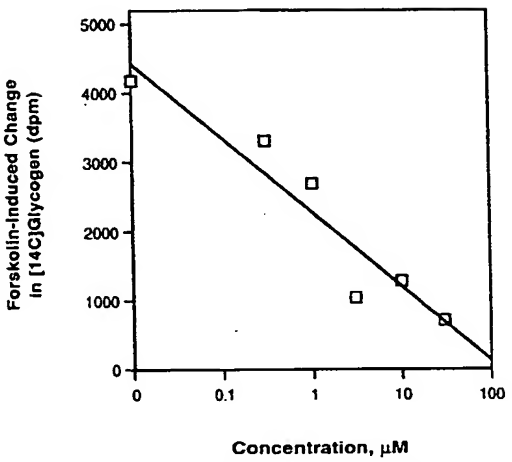


Figure 2. Difference in ^{14}C glycogen content (dpm) of [^{14}C]-glycogen prelabeled, vehicle-treated human SK-HEP-1 cells vs those treated with 50 μM forskolin (4 h incubations), at different concentrations of **1**. Mean of 3–5 experiments, each in triplicate.

Phenylalanine derivatives also have high rHLGPa inhibitory activity (Table 2). L-Phenylalanine dimethylamide **14** retained the potency of **1**, inhibiting rHLGPa with an IC_{50} of 82 nM. In contrast to the trend observed for stereoisomers of **1**, D-phenylalanine **15** (260 nM) was only about 3-fold less active than its enantiomer **14**. Similar moderate dependence of rHLGPa inhibitory activity on absolute configuration also prevailed between the enantiomeric monomethylamides **16** and **17**. Potency was retained in methyl ester **24** (140 nM) and diminished in acid **25** (1700 nM) and in the corresponding alcohol **26** (9300 nM). Changing the indole 5-substituent in the phenylalanines reduced inhibitory activity in the order Cl ≈ Br > F ≈ H > OCH_3 (analogues **14**, **18**–**21**, respectively). Thus, in the β-amino-α-hydroxybutyric acid and phenylalanine series, potency was affected very differently by changes in stereochemistry and C-terminal amide functionality, but similarly by changes to the indole 5-substituent. These results suggest that compounds in both series inhibit rHLGPa at the same site, with the 1*H*-indole-2-carboxamide moiety making a significant contribution to binding affinity. Sharply reduced activity of *N*-methylindole **37** and benzofuran **38** (rHLGP*a* IC_{50} > 10 μM) is consistent with this hypothesis. Structural and kinetic experiments are being performed to understand the inhibition mechanisms of these compounds.

To evaluate the activity of inhibitors in cells, an assay was established in a human liver-derived tissue culture cell line. In the absence of inhibitor, addition of forskolin to SK-HEP-1 cells activated glycogen phosphorylase (phosphorylation of HLGP*b* to HLGP*a*), resulting in degradation of ^{14}C-labeled glycogen (Figure 2). Inhibitor **1** blocked the forskolin effect with an IC_{50} of 1.5 μM (concentration-dependent), a value similar to that previously obtained in primary cultures of isolated human hepatocytes (IC_{50} = 2.1 μM).[18] Likewise, inhibitor **23** showed IC_{50} values of 1.9 μM (SK-HEP-1, Table 2) and 2.7 μM (human hepatocytes). Other analogues of **1** in both series are also potent inhibitors of glycogenolysis in SK-HEP-1 cells (Tables 1 and 2). These data indicate that **1** and analogues readily penetrate liver cells. Cell potency often correlated with potency in the purified enzyme assay (e.g., the two pairs of phenylalanine enantiomers **14**–**17**). Certain potent inhibitors, however, were less effective inhibitors of glycogenolysis in cells (cf. **7**, **8**, **11**, **24**). These data do not reveal factors responsible for these differences.

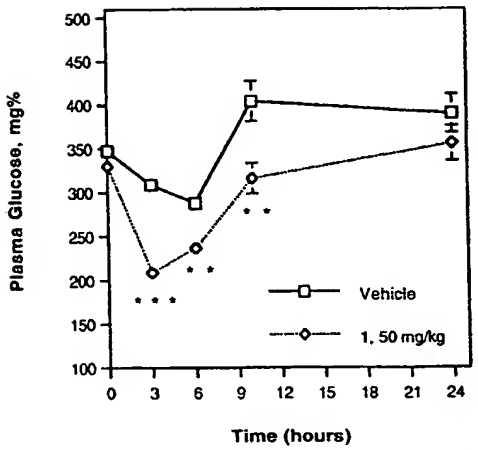


Figure 3. Time course of glucose lowering following oral dosing of **1** in diabetic *ob/ob* mice. Vertical lines show SEM. Each point represents 35–66 mice per group. Significance (***P < 0.001, **P < 0.01) by unpaired t-test relative to vehicle control groups. Similar results were obtained in an independent experiment of the same design.

Table 3. Hypoglycemic Responses Resulting from Oral Dosing of Glycogen Phosphorylase Inhibitors in Diabetic *ob/ob* Mice

compd	doses inactive, mg/kg po	dose active,[a] mg/kg po	% glucose lowering at active doses[b]	significance (P value)
1	15, 10	50[18]	40	0.05
		25[18]	32	0.05
4	50			
5	10	25	33	0.05
6	50			
10	50			
16	25	50	49	0.01
17	25	50	39	0.05
18	50			
19	50			
20	25	50	40	0.01
22	10	25	36	0.05
23	5	50	44	0.001
		25	45	0.001
		15	26	0.01
		10	24	0.05
24	50			

[a] Significant ($P \leq 0.05$) plasma glucose lowering at 3 h postdose, relative to vehicle treated controls (n = 10 per group), in drug treated *ob/ob* mice. Statistical analysis by two-tailed, unpaired Student t-test. [b] Percent decrease in drug-treated plasma glucose concentration, relative to the vehicle-treated control within the experiment.

Oral administration of **1** to diabetic (*ob/ob*) mice in the fed state at 50 or 25 mg/kg lowers plasma glucose 3 h later.[18] The time course of this effect was determined using a 50 mg/kg po dose of **1** (Figure 3). Glucose lowering was statistically significant at 3, 6, and 10 h, but not at 24 h, with the largest drop occurring at 3 h. Analogues of **1** were screened for oral activity at this timepoint (Table 3). The (2*S*) stereoisomer of **1** (**4**, not a potent rHLGP*a* inhibitor) did not show oral hypoglycemic activity at 50 mg/kg, which is consistent with phosphorylase inhibition being the mechanism of the

hypoglycemic action of **1** in vivo. Like **1**, fluoro analogue **5** also showed oral activity at 25 mg/kg, but not at 10 mg/kg. Bromoindole **6** and the methyl ester **10**, both potent inhibitors in vitro, did not show oral activity at 50 mg/kg. Several phenylalanines also showed oral activity. Hydroxypiperidines **22** and **23** were active at 25 mg/kg, and unlike **1**, **23** produced marked glucose lowering at 10 mg/kg. Furthermore, under these same conditions **23** did not alter plasma insulin levels (222 ± 26 microunits/mL, vehicle-treated versus 234 ± 32 microunits/mL, **23**-treated). Based on this finding, coupled with our previous observation that **1** did not affect insulin secretion from isolated rat islets,[18] we conclude that the observed glucose-lowering appears to be directly due to hepatic glycogenolysis inhibition and cannot be attributed to a mild insulin secretory activity. Additional experiments are being performed to better understand the origin of in vivo potency differences in these series.

Important questions arise when this approach is considered for antidiabetic therapy. Two are whether the inhibitor effects can be confined to the liver and whether glycogen will accumulate to unacceptably high levels as in glycogen storage diseases. Experiments to address these questions have given promising results. IC_{50} values for inhibition of the human muscle isoform of glycogen phosphorylase (rHMGP*a*)[37] were 200 ± 7 nM (**1**), 180 ± 22 nM (**14**), and 430 ± 15 nM (**15**), which are 2−3-fold higher than for rHLGP*a*. Additionally, compound **23** is a potent inhibitor of the muscle enzyme (83 ± 3 nM), but did not substantially impair glycogen mobilization in muscle, in situ, under conditions where liver glycogenolysis should be inhibited.[38] The absence of a muscle effect may be a result of low inhibitor concentration in muscle. Another question is whether glucose lowering can be sustained chronically by this mechanism. To this end, we have observed that **23** produces glucose lowering in diabetic *ob/ob* mice after daily b.i.d. dosing for up to 2 weeks.[38]

In summary, two series of indole-2-carboxamides derived from **1** contain potent inhibitors of human liver glycogen phosphorylase which are active in cells, and produce hypoglycemic activity on oral administration in a rodent model of type 2 diabetes. Compound **23** (CP-320626) produced oral activity at 10 mg/kg. These compounds should enable further characterization of the role of glycogenolysis in normal and disease states, and determination of the potential of glycogen phosphorylase as a therapeutic target in type 2 diabetes.

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Supporting Information Available: Synthetic procedures and characterization data for all compounds, chiral HPLC method, and biological procedures (28 pages). Ordering information is given on any current masthead page.

References

(1) Kenny, S. J.; Aubert, R. E.; Geiss, L. S. In *Diabetes in America*, 2nd ed.; Harris, M., Ed.; NIH Publication 95-1468; National Institutes of Health: Bethesda, MD, 1995; pp 47−67.
(2) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group [see comments]. *N. Engl. J. Med.* **1993**, *329*, 977−86.
(3) Abraira, C.; Colwell, J. A.; Nuttall, F. Q.; Sawin, C. T.; Nagel, N. J.; Comstock, J. P.; Emanuele, N. V.; Levin, S. R.; Henderson, W.; Lee, H. S. Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). Results of the feasibility trial. Veterans Affairs Cooperative Study in Type II Diabetes. *Diabetes Care* **1995**, *18*, 1113−23.
(4) Yki-Jarvinen, H. The liver as a target for therapy in noninsulin dependent diabetes mellitus. *Diab. Nutr. Metab.* **1994**, *7*, 109−119.
(5) Barrett, E. J.; Liu, Z. Hepatic glucose metabolism and insulin resistance in NIDDM and obesity. *Baillieres Clin. Endocrinol. Metab.* **1993**, *7*, 875−901.
(6) Consoli, A. Role of liver in pathophysiology of NIDDM. *Diabetes Care* **1992**, *15*, 430−41.
(7) Landau, B. R.; Wahren, J.; Chandramouli, V.; Schumann, W. C.; Ekberg, K.; Kalhan, S. C. Contributions of gluconeogenesis to glucose production in the fasted state. *J. Clin. Invest.* **1996**, *98*, 378−85.
(8) For a detailed discussion, see: Board, M.; Johnson, L. N. Effects of N-Acetyl-β-D-glucopyranosylamine on glycogen metabolism by isolated hepatocytes. *Diabetes Res.* **1995**, *28*, 95−109.
(9) Cori, C. F.; Cori, G. T. Mechanism of formation of hexosemonophosphate in muscle and isolation of a new phosphate ester. *Proc. Soc. Exp. Biol. Med.* **1936**, *34*, 702−705.
(10) Newgard, C. B.; Hwang, P. K.; Fletterick, R. J. The family of glycogen phosphorylases: structure and function. *Crit. Rev. Biochem. Mol. Biol.* **1989**, *24*, 69−99.
(11) Hers, H. Etudes enzymatiques sur fragments hepatiques; application a la classification des glycogenoses. *Rev. Int. Hepatol.* **1959**, *9*, 35−55.
(12) Gorin, F. A.; Mullinax, R. L.; Ignacio, P. C.; Neve, R. L.; Kurnit, D. M. McArdle's & Hers' diseases: glycogen phosphorylase transcriptional expression in human tissues. *J. Neurogenet.* **1987**, *4*, 293−308.
(13) van den Berghe, G. The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. *J. Inherit. Metab. Dis.* **1991**, *14*, 407−20.
(14) Puhakainen, I.; Koivisto, V. A.; Yki-Jarvinen, H. No reduction in total hepatic glucose output by inhibition of gluconeogenesis with ethanol in NIDDM patients. *Diabetes* **1991**, *40*, 1319−27.
(15) Rothman, D. L.; Magnusson, I.; Katz, L. D.; Shulman, R. G.; Shulman, G. I. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR. *Science* **1991**, *254*, 573−6.
(16) Magnusson, I.; Rothman, D. L.; Katz, L. D.; Shulman, R. G.; Shulman, G. I. Increased rate of gluconeogenesis in type II diabetes mellitus. A ^{13}C nuclear magnetic resonance study. *J. Clin. Invest.* **1992**, *90*, 1323−7.
(17) Hellerstein, M. K.; Neese, R. A.; Linfoot, P.; Christiansen, M.; Turner, S.; Letscher, A. Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans. A stable isotope study. *J. Clin. Invest.* **1997**, *100*, 1305−1319.
(18) Martin, W. H.; Hoover, D. J.; Armento, S. J.; Stock, I. A.; McPherson, R. K.; Danley, D. E.; Stevenson, R. W.; Barrett, E. J.; Treadway, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1776−1781.
(19) Shiota, M.; Jackson, P. A.; Bischoff, H.; McCaleb, M.; Scott, M.; Monohan, M.; Neal, D. W.; Cherrington, A. D. Inhibition of glycogenolysis enhances gluconeogenic precursor uptake by the liver of conscious dogs. *Am. J. Physiol.* **1997**, *273*, E868−E879.
(20) Sprang, S. R.; Withers, S. G.; Goldsmith, E. J.; Fletterick, R. J.; Madsen, N. B. Structural basis for the activation of glycogen phosphorylase *b* by adenosine monophosphate. *Science* **1991**, *254*, 1367−71.
(21) Johnson, L. N.; Snape, P.; Martin, J. L.; Acharya, K. R.; Barford, D.; Oikonomakos, N. G. Crystallographic binding studies on the allosteric inhibitor glucose-6-phosphate to T state glycogen phosphorylase *b*. *J. Mol. Biol.* **1993**, *232*, 253−67.
(22) Oikonomakos, N. G.; Zographos, S. E.; Johnson, L. N.; Papageorgiou, A. C.; Acharya, K. R. The binding of 2-deoxy-D-glucose 6-phosphate to glycogen phosphorylase *b*: kinetic and crystallographic studies. *J. Mol. Biol.* **1995**, *254*, 900−17.

(23) Zographos, S. E.; Oikonomakos, N. G.; Tsitsanou, K. E.; Leonidas, D. D.; Chrysina, E. D.; Skamnaki, V. T.; Bischoff, H.; Goldmann, S.; Watson, K. A.; Johnson, L. N. The structure of glycogen phosphorylase *b* with an alkyl-dihydropyridine-dicarboxylic acid compound, a novel and potent inhibitor. *Structure (London)* **1997**, *5*, 1413−1425.
(24) For leading references, see: De la Fuente, C.; Krulle, T. M.; Watson, K. A.; Gregoriou, M.; Johnson, L. N.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Fleet, G. W. J. Glucopyranose spirohydantoins. Specific inhibitors of glycogen phosphorylase. *Synlett* **1997**, 485−487.
(25) Kasvinsky, P. J.; Shechosky, S.; Fletterick, R. J. Synergistic regulation of phosphorylase *a* by glucose and caffeine. *J. Biol. Chem.* **1978**, *253*, 9102−6.
(26) Watson, K. A.; Mitchell, E. P.; Johnson, L. N.; Son, J. C.; Bichard, C. J. F.; Orchard, M. G.; Fleet, G. W. J.; Oikonomakos, N. G.; Leonidas, D. D.; Kontou, M.; Papageorgioui, A. Design of inhibitors of glycogen phosphorylase: a study of α- and β-C-glucosides and 1-thio-β-D-glucose compounds. *Biochemistry* **1994**, *33*, 5745−58.
(27) Krulle, T. M.; Watson, K. A.; Gregoriou, M.; Johnson, L. N.; Crook, S.; Watkin, D. J.; Griffiths, R. C.; Nash, R. J.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Fleet, G. W. J. Specific inhibition of glycogen phosphorylase by a spirodiketopiperazine at the anomeric position of glucopyranose. *Tetrahedron Lett.* **1995**, *36*, 8291−4.
(28) Oikonomakos, N. G.; Kontou, M.; Zographos, S. E.; Watson, K. A.; Johnson, L. N.; Bichard, C. J. F.; Fleet, G. W. J.; Acharya, K. R. *N*-acetyl-β-D-glucopyranosylamine: A potent T-state inhibitor of glycogen phosphorylase. A comparison with α-D-glucose. *Protein Sci.* **1995**, *4*, 2469−77.
(29) Mitchell, E. P.; Withers, S. G.; Ermert, P.; Vasella, A. T.; Garman, E. F.; Oikonomakos, N. G.; Johnson, L. N. Ternary complex crystal structures of glycogen phosphorylase with the transition state analog nojirimycin tetrazole and phosphate in the T and R states. *Biochemistry* **1996**, *35*, 7341−7355.
(30) Kasvinsky, P. J.; Madsen, N. B.; Sygusch, J.; Fletterick, R. J. The regulation of glycogen phosphorylase *a* by nucleotide derivatives. Kinetic and x-ray crystallographic studies. *J. Biol. Chem.* **1978**, *253*, 3343−51.
(31) Ercan-Fang, N.; Nuttall, F. Q. The effect of caffeine and caffeine analogs on rat liver phosphorylase *a* activity. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 1312−8.
(32) Ercan, N.; Gannon, M. C.; Nuttall, F. Q. Allosteric regulation of liver phosphorylase *a*: revisited under approximated physiological conditions. *Arch. Biochem. Biophys.* **1996**, *328*, 255−64.
(33) Newgard, C. B.; Nakano, K.; Hwang, P. K.; Fletterick, R. J. Sequence analysis of the cDNA encoding human liver glycogen phosphorylase reveals tissue-specific codon usage. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8132−6.
(34) Parris, K. D.; Hoover, D. J.; Damon, D. B.; Davies, D. R. Synthesis and crystallographic analysis of two rhizopuspepsin inhibitor complexes. *Biochemistry* **1992**, *31*, 8125−41.
(35) Brook, M. A.; Chan, T. H. A simple procedure for the esterification of carboxylic acids. *Synthesis* **1983**, 201−3.
(36) Dugger, R. W.; Ralbovsky, J. L.; Bryant, D.; Commander, J.; Massett, S. S.; Sage, N. A.; Selvidio, J. R. A novel synthesis of nor-C-statine. *Tetrahedron Lett.* **1992**, *33*, 6763−6.
(37) Carty, M. D.; Nelson, R. T.; Soeller, W. C. Unpublished results. A full-length human muscle glycogen phosphorylase cDNA was obtained by RT-PCR using a human muscle cDNA library, then subcloned into a pKK223−3 vector, and expressed in E. coli as described by Coats, W. S.; Browner, M. F.; Fletterick, R. F.; Newgard, C. B. *J. Biol. Chem.* **1991**, *266*, 16113−9. The sequence has been submitted to GenBank, Accession No. AF066859.
(38) Gibbs, E. M.; Treadway, J. L. Unpublished results, manuscript in preparation.

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